STUDY TITLE

Benchmark Assessment of Corn Rootworm Susceptibility to Event DAS-59122-7 Using the Sub-Lethal Seedling Assay (SSA): 2009 Growing Season

DATA REQUIREMENTS

None

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STUDY COMPLETION DATE

December, 1 2010

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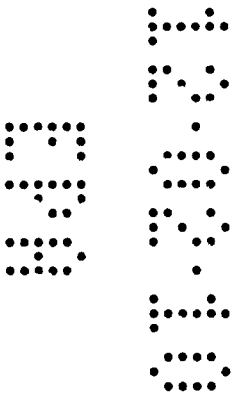
PHI-2010-241



STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the Federal Insecticide, Fungicide, and Rodenticide Act, (FIFRA) Section 10(d)(1)(A), (B), or (C).*

Company: Pioneer Hi-Bred International, Inc.

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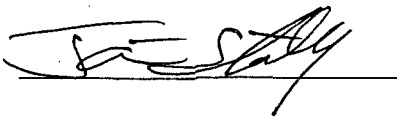
Signature: 

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* In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was not conducted in accordance with the requirements for the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR 160, 1989.

12-01-2010

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CERTIFICATION PAGE

We, the undersigned, declare that this report accurately represents the results observed during the course of this study.

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TABLE OF CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT 3

CERTIFICATION PAGE 4

TABLE OF CONTENTS 5

ABSTRACT 6

INTRODUCTION 7

METHODS 7

Table 1. Converting Corn Rootworm Head Capsule Width to Instar 9

RESULTS 10

Table 2. Instar Distribution and Odds Ratio (Proportion of 3rd Instars) of U.S. Western Corn Rootworm Populations Collected in 2009 12

Table 3. Image Data (Body Area: mm2) of U.S. Western Corn Rootworm Populations Collected in 2009

Table 4. Instar Distribution and Odds Ratio (Proportion of 3rd Instars) of U.S. Northern Corn Rootworm Populations Collected in 2009 14

Table 5. Image Data (Body Area: mm2) of U.S. Northern Corn Rootworm Populations Collected in 2009 14

DISCUSSION 15

REFERENCES 16

ARCHIVING STATEMENT 16

ABSTRACT

Optimum® AcreMax™ 1 Insect Protection (OAM1) and Optimum® AcreMax™ RW Insect Protection (OAMRW) registration extensions were granted by the U.S. Environmental Protection Agency on September 29, 2010 (EPA Reg. No: 29964-6 and 29964-10). OAM1 is a blended seed product that contains 90% Herculex® Xtra Insect Protection (Cry1F x Cry34/35Ab1) and 10% Herculex® I Insect Protection (Cry1F). OAMRW is a blended seed product that contains 90% Herculex® Rootworm Insect Protection (Cry34/35Ab1) and 10% non-*Bt* corn. The Cry34/35Ab1 *Bt* insecticidal binary protein expressed in corn event 59122 provides protection from corn rootworm (CRW, *Diabrotica* spp.) feeding damage. As a condition of registration, Pioneer is required to implement a detailed OAM1 and OAMRW-specific resistance monitoring and remedial action plan, including western corn rootworm (WCR, *Diabrotica virgifera virgifera*) baseline (benchmark) susceptibility to 59122 using the Sub-lethal Seedling Assay (SSA). The interim CRW resistance monitoring plan using the SSA is outlined in this report and includes the following: sampling plan, bioassay methodology, initial CRW sensitivity studies, and an interim description of the process for developing rootworm suspected resistance guidelines based on SSA data.

INTRODUCTION

Optimum® AcreMax™ 1 Insect Protection (OAM1) and Optimum® AcreMax™ RW Insect Protection (OAMRW) registration extensions were granted by the U.S. Environmental Protection Agency on September 29, 2010 (EPA Reg. No: 29964-6 and 29964-10). OAM1 is a blended seed product that contains 90% Herculex® Xtra Insect Protection (Cry1F x Cry34/35Ab1) and 10% Herculex® I Insect Protection (Cry1F). OAMRW is a blended seed product that contains 90% Herculex® Rootworm Insect Protection (Cry34/35Ab1) and 10% non-*Bt* corn. The Cry34/35Ab1 *Bt* insecticidal binary protein expressed in corn event 59122 provides protection from corn rootworm (CRW, *Diabrotica* spp.) feeding damage. As a condition of registration, Pioneer is required to implement a detailed OAM1 and OAMRW-specific resistance monitoring and remedial action plan, including western corn rootworm (WCR, *Diabrotica virgifera virgifera*) baseline (benchmark) susceptibility to DAS-59122-7 (hereafter referred to as 59122) using the Sub-lethal Seedling Assay (SSA).

The interim CRW resistance monitoring plan using the SSA is outlined in this report and includes the following: sampling plan, bioassay methodology, initial CRW sensitivity studies, and an interim description of the process for developing rootworm suspected resistance guidelines based on SSA data.

METHODS

WESTERN CORN ROOTWORM POPULATION COLLECTIONS

Thirteen populations of WCR adults were collected in 2009 as part of the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) sampling program for resistance monitoring. The collection sites represented the areas of the US Corn Belt where this species is most abundant, and included populations from three regions. ABSTC *Region 1* consisted of Illinois and Indiana and includes the rotation-resistant variant (soybean biotype); *Region 2* consisted of Iowa and Missouri and includes the wild type; and *Region 3* consisted of Nebraska and Kansas and includes the organophosphate resistant variant. Additionally, two WCR and one northern corn rootworm (NCR, *Diabrotica barberi*) field populations were collected from Iowa in 2009 by Pioneer and were included in this study. Each collection consisted of at least 2000 adults and was maintained for oviposition purposes by Custom Bio-Products (Maxwell, IA). Eggs from a laboratory population of diapausing WCR from the USDA-ARS North Central Agricultural Research Laboratory (Brookings, SD) were included as a susceptible reference. Therefore, a total of 17 populations (16 WCR and one NCR) were assayed using the SSA in 2010. Diapausing eggs from all populations were sent to Pioneer for SSA bioassays. The populations collected in 2009 were located in the following counties:

ABSTC *Region 1 (rotation resistant variant)*

- Champaign County, IL
- McLean County, IL
- Iroquois County, IL
- Ford County, IL
- Peoria County, IL
- Bureau County, IL

ABSTC *Region 2 (wild type)*

- Story County, IA
- Franklin County, IA
- Scott County, IA

- Boone County, IA (collected by Pioneer)
- Delaware County, IA (WCR) (collected by Pioneer)
- Delaware County, IA (NCR) (collected by Pioneer)

ABSTC *Region 3 (organophosphate resistant variant)*

- Howard County, NE
- Sherman County, NE
- Hamilton County, NE
- Clay County, NE

Because populations included in this report were collected in the summer of 2009 and the ABSTC collections only accounted for *Regions 1-3* listed above, populations from *Region 4* (Minnesota, South Dakota, and Wisconsin, as listed by EPA in the terms and conditions of OAM1 and OAMRW registrations) were not included in the 2009 benchmark assessment. However, populations were collected from *Region 4* in 2010.

BIOASSAY METHODOLOGY AND STANDARD PROCEDURES

A. Seedling Assay

The SSA consists of exposing eggs from CRW populations to either 59122 or near isoline (non-*Bt*) corn seedlings. The containers of infested seedling mats were maintained under controlled environmental conditions for a precise period of time to maximize the potential for sub-lethal effects to CRW larvae but extracted prior to pupation. Larvae were extracted from the seedling mats and developmental effects were evaluated. The test system is described below.

Seedling assays were conducted using eggs from each of the field populations collected in 2009 and the susceptible laboratory USDA colony (Brookings, SD). Prior to infestation, eggs were incubated in top soil for 10 days at 25°C. Eggs were washed from the soil using a series of sieves and suspended in 0.08% agar. Assay container setup and infestation occurred 3-7 days prior to initial egg hatch depending on the population. The test system utilized 10 ¾ x 9 ¼ x 3 ½ inch (L x W x H) clear plastic containers with hinged lids (Clear-View SmartLock Hinged Lock Containers, CI8-3035; Pactiv Corp., Lake Forest, IL). Container setup involved placing 150 kernels of corn seed treated with fungicidal seed treatment in the bottom of each container. Three replicates (containers), each of 59122 and negative control (isoline) were setup per population. Two hundred (200) ml of a 1% fungicide solution (3336F Turf and Ornamental Systemic Fungicide; Cleary Chemical, Dayton, NJ) was added to each container. The containers were then filled with 700 ml of dry Sunshine MVP Professional Growing Mix (Sun Gro Horticulture Ltd., Vancouver BC). Immediately after adding the soil, the pre-incubated CRW eggs suspended in agar were dispensed at a rate of approximately 1,000 eggs/container onto the soil surface. The infested containers were then closed and placed in an environmental chamber set at 25°C, 65% relative humidity (RH), and a photoperiod cycle of 14:10 hours light: dark. On or near the date that first egg hatch was observed in the hatch test for a given population, 9 evenly spaced 10 mm holes were punched in the lid for ventilation.

Hatch tests were set up for each population on the same date containers were assembled. Hatch tests were made by dispensing a minimum of 90 eggs suspended in agar to the surface of 3 Petri dishes (~30 eggs/dish) filled with moistened sifted topsoil (80-mesh sieve). The Petri dishes were then sealed with Parafilm, placed near the containers in the environmental chamber, and monitored daily for initial hatch. Final hatch rate for each population was recorded upon completion of hatch.

Larvae were allowed to feed and develop in the containers for 17 days at 25°C (~495 heat units) after initial egg hatch. The "seedling mats" were then removed from their respective containers and each placed in a separate Burlese funnel for larval extraction. Larvae were extracted into 70% ethanol using low-wattage (15-watt) light bulbs in the funnels. The extraction process was allowed to run for a minimum of 3 days to ensure complete extraction.

B. Sample Processing

Upon completion of extraction, larvae were measured using 2 separate procedures. In the first procedure, each sample was poured into a sampling pan with equal-sized and numbered grid squares in a manner that evenly distributed the larvae across the pan. All larvae were then counted and a random sub-sample of 30 larvae was removed from the pan. This sub-sample was selected using a set of random numbers to pick one of the grid-squares as a starting point and sampling all the larvae contained within that grid-square. This process was repeated until a total of 30 larvae were sampled. Larvae from the subsample were categorized into one of three instars by measuring the width of their head capsule under a dissecting microscope. Head capsule widths were measured using an ocular grid (10x10 mm; 0.5 mm divisions) placed in the eyepiece of the microscope at a magnification of 64x. Each larva was placed as flat as possible on the microscope stage (ventral side down) and the widest point of the head capsule measured. Each head capsule width measurement was rounded to the nearest ½ grid when recording. Table 1 contains rootworm head capsule width data published by Hammack et al. (2003), and was used to convert head widths measured in "grid-squares" to instar. The same conversion factors were used for both northern and western corn rootworm.

Table 1. Converting Corn Rootworm Head Capsule Width to Instar

Instar	Width (μm)	Width (inches)	Width (grids)
First	< 270	< 0.011	< 3.4
Second	270 – 410	0.011 – 0.016	3.43 – 5.20
Third	> 410	> 0.016	> 5.20

The second procedure involved pouring each sample into a large Petri dish which was then placed on a flatbed scanner (Perfection V500 Photo, J251A, EPSON America, Inc., Long Beach, CA) for image capture. The resulting images were imported into image analysis software (Image Pro Plus v.7.0, Media Cyber Genetics Inc., Bethesda, MD) which measured the total body area of each insect in pixels and converted the area to mm^2.

C. Statistical Analysis

The experimental design included two treatments, 59122 and isoline (non-*Bt* negative control), arranged in a randomized complete block design (RCBD) with three blocks (i.e. replicates), with container as the experimental unit. Each replicate was infested with approximately 1,000 eggs, for a total of approximately 3,000 eggs tested in each seedling type per population. All three replicates of both treatments within each population were conducted at the same time and under the same conditions.

The response of the CRW populations on the isoline root mats was used to account for possible differences in development across populations assayed at different times due to environmental effects (e.g. variation in temperature, humidity, time of assay, time of egg hatch).

Instar Data

For each experimental unit, the response is the number of larvae in each of three potential instars and such response follows a multinomial distribution with three categories. A generalized linear mixed model assuming multinomial distribution and using cumulative logit function was employed to analyze the response using SAS PROC GLIMMIX. According to the design for the benchmark experiment, 'population', 'treatment' and 'rep' were factors included in the statistical model. The proportion (i.e. percentage) of larvae in each of the three instars was estimated for each treatment within each population across three replicates from the statistical model. In addition, a statistical comparison was made between the two treatments within each population to compare the difference in age distribution between larval populations exposed to seedling mats containing 59122 and isoline. The result of each comparison is expressed as the odds ratio between the two treatments (59122: isoline). The odds ratio is a measurement of the magnitude of the difference in larval development rate between those exposed to 59122 seedlings and isoline seedlings. Odds ratios less than 1.0 indicate larvae exposed to 59122 seedlings developed more slowly than those exposed to isoline seedlings, and the smaller the odds ratio, the greater the effect. In contrast, odds ratios equal or greater than 1.0 mean larvae exposed to 59122 seedlings developed equally fast or faster than those exposed to isoline seedlings.

Image Data

For each experimental unit, the response is the body size of individual larvae (measured as "area") and such response follows a normal distribution. A linear mixed model assuming a normal distribution was employed using SAS PROC MIXED. According to the design for the benchmark experiment, 'population', 'treatment' and 'rep' were factors included in the statistical model. Mean area of larvae was estimated for each treatment within each population across three replicates from the statistical model. In addition, a statistical comparison was made between the two treatments within each population to compare the difference in body size between larval populations being exposed to seedling mats containing 59122 and isoline. The result of each comparison is expressed as the difference between the two treatments (59122 - isoline).

Suspected Resistance based on SSA Data

Resistance will be suspected when a population responds similarly when exposed to both 59122 and isoline. In this case, resistance is identified if there is no difference between 59122 and isoline treatment groups within the same population. Based on the current statistical analysis, this is identified when the odds ratio of instar development rate (instar data) between 59122 and the isoline treatments is equal or greater than 1.0 and/or the difference in larval body size (image data) between 59122 and isoline is equal or greater than 0.

RESULTS

Sub-lethal seedling assays were conducted on 15 WCR and one NCR field populations collected in 2009, and the WCR laboratory susceptible reference colony. CRW population development was evaluated using two methods: 1) instar distribution based on head capsule measurement (Hammack et al. 2003) on a sub-sample targeting 30 individuals per replication (maximum of 90 measurements per population per treatment) and, 2) using an image (body size) data collection method that allows for the inclusion of all individuals recovered from the assay in the statistical analysis. Not all populations generated replicates that allowed for random evaluation of head capsule from 30 individuals per replicate (see Table 2, N<90), in which case all individuals extracted were included in the analysis. Populations that did not meet the minimum sample size criteria in both treatments were Boone (IA), Brookings (SD), Bureau (IL), Iroquois (IL), and Scott (IA) (Table 2). Additional populations that did not reach the minimum sample size criteria for either 59122 or the isoline were Clay (NE), Delaware (IA), Hamilton (NE), Mclean (IL), Story (IA), and Ford (IL).

Results from WCR instar comparisons (using head capsule measurement) expressed as the odds ratio between the two treatments (59122: isoline) within a population and their associated p-values are shown in Table 2. The average instar distribution of WCR across all field populations exposed to isoline (non-*Bt*) was 0.77%, 15.99%, and 83.25% for first, second, and third instars, respectively. There was a significant shift in instar distribution when larvae were exposed to 59122 seedling mats, with an average of 4.81%, 53.85%, and 41.33% of first, second, and third instars, respectively, across all populations. The observed shift to earlier instars on 59122 indicates a measure of efficacy provided by the trait. Instar distribution for the Brookings (SD) laboratory susceptible reference strain was 0.1%, 6.0% and 93.9% for isoline and 5.3%, 60.5%, and 34.2% for 59122 (first, second, and third instars, respectively). Instar distribution was variable across populations within treatment, ranging from 17.60% (Mclean, IL) to 61.30% (Delaware, IA) for third instars on 59122, and 57.30% (Sherman, NE) to 100% (Bureau, IL) for third instars on isoline (Table 2). However, all odds ratios for all populations were below 1.0 and were statistically significant at 0.05 level (p-value < 0.05; odds ratio of 1.0 indicates that instar distribution for the 59122 and isoline treatments are the same; odds ratio < 1 indicates CRW larval development was delayed when exposed to 59122, which is an indication of efficacy provided by the trait).

Results from WCR image comparisons expressed as the mean body size difference between the two treatments (59122 - isoline) within each population and their associated p-values are shown in Table 3. The average mean larval size was 2.49mm^2 (ranging from 2.0 to 3.4mm^2) and 4.66mm^2 (ranging from 3.7 to 5.2mm^2) for the 59122 and isoline treatments, respectively, across all populations (Table 3). Mean differences between treatments within populations averaged -2.17, with a range from -3.1 (Champaign, IL) to -1.4 (Iroquois, IL) (Table 3). Mean size differences for all populations were below 0 and were statistically significant (p-value < 0.05), which indicates that WCR population size on the isoline treatment was always significantly higher than that on 59122.

Results from NCR instar and image comparisons are shown in Tables 4 and 5, respectively. The developmental rate of the NCR population based on head capsule measurement was significantly reduced when exposed to 59122 when compared to the isoline treatment, as shown by the proportion of 3rd instars in each treatment (43.5% and 82.4% on 59122 and isoline, respectively (p-value < 0.0001) (Table 4). Image data showed a similar response, with a mean body size difference between 59122 and isoline treatments of -1.5 (p-value < 0.0001) (Table 5).

Table 2. Instar Distribution and Odds Ratio (Proportion of 3rd Instars) of U.S. Western Corn Rootworm Populations Collected in 2009

Population	59122 [a]				Isoline (non-*Bt* control) [a]				Odds Ratio [c] (*Bt*):(non-*Bt*)	p-value
	N[b]	1st instar	2nd instar	3rd instar	N[b]	1st instar	2nd instar	3rd instar		
Boone, IA	17	1.6%	33.6%	64.8%	87	0.3%	10.1%	89.6%	0.21	0.0122
Brookings, SD [d]	88	5.3%	60.5%	34.2%	81	0.1%	6.0%	93.9%	0.03	<.0001
Bureau, IL	62	2.0%	39.2%	58.8%	68	0.0%	0.0%	100.0%	0.00	na [e]
Champaign, IL	90	8.7%	68.2%	23.1%	90	0.7%	17.4%	81.9%	0.07	<.0001
Clay, NE	80	4.9%	58.9%	36.2%	90	0.1%	5.4%	94.5%	0.03	<.0001
Delaware, IA	82	1.8%	36.9%	61.3%	90	0.2%	6.4%	93.4%	0.11	<.0001
Ford, IL	90	2.9%	48.3%	48.8%	88	1.5%	32.5%	66.0%	0.49	0.0190
Franklin, IA	90	3.3%	51.1%	45.6%	90	0.3%	8.6%	91.1%	0.08	<.0001
Hamilton, NE	84	7.4%	65.9%	26.7%	90	0.6%	16.0%	83.4%	0.07	<.0001
Howard, NE	90	6.6%	64.5%	28.9%	90	0.4%	10.7%	88.9%	0.05	<.0001
Iroquois, IL	55	2.7%	46.9%	50.4%	88	0.3%	9.9%	89.8%	0.12	<.0001
Mclean, IL	82	11.9%	70.5%	17.6%	90	3.9%	54.1%	42.0%	0.29	0.0003
Peoria, IL	90	3.7%	53.4%	42.9%	90	0.7%	18.1%	81.2%	0.17	<.0001
Scott, IA	52	3.1%	49.6%	47.3%	76	0.1%	2.5%	97.4%	0.02	<.0001
Sherman, NE	90	7.8%	66.7%	25.5%	90	2.1%	40.6%	57.3%	0.25	<.0001
Story, IA	80	3.8%	54.1%	42.1%	90	0.3%	7.5%	92.2%	0.06	<.0001

[a] Data generated using head capsule measurements.
[b] N = sum of larvae evaluated over the three replicates. Not all populations generated replicates that allowed for random evaluation of head capsule from 30 individuals per replicate (N<90), in which case all individuals extracted were included in the analysis.
[c] dds ratio significantly lower than 1.0 indicates that instar distribution between 59122 and isoline are different and insects exhibited significant developmental delay upon exposure to 59122.
[d] Brookings, SD was included in the assay as a laboratory 59122 susceptible reference population.
[e] p-value is not available for Odds Ratio test due to lack of variation in one treatment group: 100% of larvae in the non-*Bt* control group developed to the 3rd instar. Using Fisher's Exact test the proportion of 3rd instars was significantly different between treatments for this population (p-value <.0001).

Table 3. Image Data (Body Area: mm²) of U.S. Western Corn Rootworm Populations Collected in 2009

Population	59122		Isoline (non-*Bt* control)		Difference [a] (*Bt*)-(non-*Bt*)	p-value
	N [b]	Mean	N [b]	Mean		
Boone, IA	18	3.0	172	4.7	-1.7	0.0025
Brookings, SD [c]	113	2.5	97	4.9	-2.4	<.0001
Bureau, IL	63	2.8	85	4.5	-1.7	<.0001
Champaign, IL	515	2.1	913	5.2	-3.1	<.0001
Clay, NE	126	2.1	613	4.3	-2.2	<.0001
Delaware, IA	111	3.4	388	5.1	-1.7	<.0001
Ford, IL	384	2.1	390	4.7	-2.6	<.0001
Franklin, IA	440	2.5	665	4.8	-2.3	<.0001
Hamilton, NE	108	2.3	510	4.6	-2.3	<.0001
Howard, NE	434	2.0	745	5.1	-3.1	<.0001
Iroquois, IL	55	2.9	241	4.3	-1.4	<.0001
Mclean, IL	193	2.4	961	4.6	-2.2	<.0001
Peoria, IL	441	2.1	407	3.7	-1.6	<.0001
Scott, IA	57	2.9	146	4.6	-1.7	<.0001
Sherman, NE	520	2.1	618	4.8	-2.7	<.0001
Story, IA	121	2.6	679	4.6	-2.0	<.0001

[a] Difference between the mean values observed for the populations feeding on 59122 and isoline.
[b] N = sum of all larvae extracted from the three replicates.
[c] Brookings, SD was included in the assay as a laboratory 59122 susceptible reference population.

Table 4. Instar Distribution and Odds Ratio (Proportion of 3rd Instars) of U.S. Northern Corn Rootworm Populations Collected in 2009

Population	59122 [a]				Isoline (non-*Bt* control) [a]				*Bt*: non-*Bt* [b] Odds Ratio	p-value
	N[c]	1st instar	2nd instar	3rd instar	N[c]	1st instar	2nd instar	3rd instar		
Delaware, IA	90	3.6%	52.9%	43.5%	90	0.6%	17.0%	82.4%	0.16	<.0001

[a] Data generated using head capsule measurements
[b] Odds ratio significantly lower than 1.0 indicates that instar distribution between 59122 and isoline are different and insects exhibited significant developmental delay upon exposure to 59122.
[c] N = sum of larvae evaluated over the three replicates (30 larvae replicate).

Table 5. Image Data (Body Area: mm²) of U.S. Northern Corn Rootworm Populations Collected in 2009

Population	59122		Isoline (non-*Bt* control)		Difference [a] (*Bt*)-(non-*Bt*)	p-value
	N[b]	Mean	N[b]	Mean		
Delaware, IA	461	2.2	732	3.7	-1.5	<.0001

[a] Difference between the mean values observed for the population feeding on 59122-7 and isoline.
[b] N = sum of all larvae extracted from the three replicates.

DISCUSSION

The CRW resistance-monitoring program for OAM1 and OAMRW using the SSA is designed to detect changes in population sensitivity to event 59122. The objective of the OAM1 and OAMRW-specific monitoring program using the SSA is to measure biologically-relevant changes in CRW sensitivity to 59122 at the population level. These changes in sensitivity can be detected through chronic exposure to 59122 in the SSA and measuring subsequent effects on parameters characterizing larval development rate. The strengths of this assay system include the ability to screen large numbers of insects at a lower cost per insect screened compared to existing bioassay methodology. The assay also provides a realistic exposure scenario by using the transformed plant and would be sensitive to all known CRW resistance mechanisms. The increased sensitivity of the SSA should allow for detection of such changes earlier in the process of resistance evolution due to its ability to detect subtle differences in CRW susceptibility (Nowatzki et al. 2008), therefore enhancing the monitoring program.

This report contains the first year of data representing the current susceptibility of U.S. field corn rootworm populations to 59122 using the SSA, prior to large-scale introduction of OAM1 and OAMRW. Evaluation of rootworm susceptibility to 59122 using the SSA was conducted using two methods: head capsule (Hammack et al. 2003), and body size measurements (image analysis). Regardless of the method used to evaluate the SSA data, all CRW populations tested showed a significant delay in developmental rate when exposed to 59122 seedling mats compared to isoline. From an efficiency perspective, the image analysis has an advantage as all individuals exposed to and recovered from the assay can be included in the analysis. In contrast, sub sampling is required for the head capsule data due to the labor involved in measurements. Both head capsule and body size data presented in this report should be interpreted as the best representation of natural variability of CRW populations' susceptibility to 59122 available using the SSA prior to large scale adoption of 59122 integrated rootworm refuge products. Pioneer will continue to investigate the use of both methods for assessing WCR susceptibility.

The statistical analyses used for the 2009 SSA data are robust and effective at measuring developmental differences between populations and between treatments within populations. For future monitoring purposes, the point at which a population is considered significantly less susceptible than the benchmark will be determined with the addition of data from subsequent benchmark populations. Pioneer will continue to develop and refine the statistical method to analyze data generated from the SSA for use in the monitoring program.

DECISION PROCESS LEADING TO A POTENTIAL REMEDIAL ACTION PLAN

Resistance will be suspected if populations screened using the SSA responds similarly when exposed to both 59122 and isoline. Procedures to confirm suspected resistance will be followed as outlined in the terms and conditions described in OAM1 and OAMRW registrations.

REFERENCES

Hammack L, Ellsbury MM, Roehrdanz RL, Pikul Jr JL, 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96, 1153-1159.

Nowatzki, T.M., S.A. Lefko, R.R. Binning, S.D. Thompson, T.A. Spencer, and B.D. Siegfried. 2008. Validation of a novel resistance monitoring technique for corn rootworm (Coleoptera: Chrysomelidae) and event DAS-59122-7 maize. Journal of Applied Entomology 132: 177-188

ARCHIVING STATEMENT

Original or exact copies of all raw data and pertinent information, including the original study plan, any amendments, and the final report will be archived at:

Pioneer Hi-Bred International, Inc.
Regulatory Group Archives
2450 SE Oak Tree Court
Ankeny, IA 50021